

Measurement of IgE antibodies to shrimp tropomyosin is superior to skin prick testing with commercial extract and measurement of IgE to shrimp for predicting clinically relevant allergic reactions after shrimp ingestion

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Background: Shrimp is a frequent cause of food allergy. Tropomyosin is the major allergen in shrimp, and it shares homology to tropomyosins from other crustaceans, dust mites, cockroach, and parasites.

Objective: The aim of this study was to determine the value of detection of IgE to shrimp tropomyosin in the diagnosis of shrimp allergy.

Methods: We have studied 35 patients with asthma, rhinitis, or both who were sensitized to *Dermatophagoides pteronyssinus*. All subjects underwent skin prick testing in addition to double-blind, placebo-controlled food challenges (DBPCFC); oral open challenges; or both with shrimp. Measurements of IgE to shrimp and shrimp tropomyosin were carried out by means of CAP and chimeric ELISA, respectively.

Results: Oral challenges confirmed the diagnosis of shrimp allergy in 7 patients. IgE measurement to shrimp tropomyosin was positive in 71.4% of the patients with shrimp allergy. Of the 28 patients without shrimp allergy, only 7.1% (2/28) had IgE to shrimp tropomyosin compared with 25% (7/28) who had IgE to shrimp and 35.7% (10/28) who had positive skin prick test responses to shrimp. Sensitivity was similar for all 3 methods (71.4%); in contrast, specificity of IgE to shrimp tropomyosin

(92.8%) was greater than that of IgE to shrimp (75%) and skin prick testing (64.2%). With regard to diagnostic efficiency, measurement of IgE to shrimp tropomyosin was superior to measurement of IgE to shrimp and skin prick testing (88.5%, 74.2%, and 65.7%, respectively).

Conclusion: Use of measurements of IgE to shrimp tropomyosin provided added value to the diagnosis of shrimp allergy. (J Allergy Clin Immunol 2010;125:872-8.)

Key words: Food allergy, shrimp, tropomyosin, food challenge, skin prick test, specific IgE

Crustaceans, including shrimp, are a frequent cause of food allergy and are capable of provoking serious and fatal reactions. The diagnostic workup for shrimp allergy includes clinical history, skin prick tests (SPTs), measurement of specific IgE levels in serum, and oral food challenges. The history might not be clear-cut in some patients, and the presence of IgE antibodies is not always associated with clinical reactions; in contrast, a few patients with a history of a positive reaction might not have detectable IgE antibodies.¹ Double-blind, placebo-controlled food challenges (DBPCFCs) are generally accepted as the gold standard² and are particularly useful to provide definitive advice to patients regarding food avoidance. However, DBPCFCs are time-consuming, costly, and can induce potentially severe clinical symptoms. Therefore it would be advantageous to have reliable diagnostic methods that would make oral food challenges unnecessary.

Previous studies have attempted to correlate levels of serum specific IgE to foods to the outcome of respective oral food challenges, and IgE decision points that would give a 95% probability of a positive DBPCFC result have been established for a few foods, including milk, egg, and peanut.³⁻⁸ However, specific IgE decision points have shown marked variations in different studies related to age and population studied, ethnicity, and type of food allergen, hampering their widespread use as accurate predictors of clinically relevant reactions to foods.⁹ More recently, the efficacy of component-resolved diagnosis with purified natural allergens, recombinant allergens, or both on microarray chips or skin testing has been demonstrated for several foods, including milk, egg, wheat, apple, hazelnut, cherry, peanut, and others, leading to improved diagnosis and, in some cases, prediction of more severe reactions.¹⁰⁻¹⁹ Similar studies focusing on shrimp allergy are few. Jirapongsananuruk et al²⁰ have recently reported that mean wheal diameters of 20 to 30 mm on skin tests performed

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
NPV: Negative predictive value
PPV: Positive predictive value
SPT: Skin prick test

with commercial and raw shrimp extracts and using the prick-to-prick approach provided 95% predictive probability of a positive DBPCFC result in patients from Thailand allergic to the black tiger prawn (*Penaeus monodon*) and the giant freshwater prawn (*Macrobrachium rosenbergii*).

At least 80% of subjects allergic to shrimp react to the major allergen tropomyosin, and up to 75% of patients' specific IgE to shrimp might be directed to this molecule.²¹ There is a high degree of sequence identity among tropomyosins from invertebrates, including shrimp and other crustaceans, dust mites, cockroach, and parasites, with evidence for immunologic cross-reactivity.²²⁻²⁴ The clinical relevance of sensitization to tropomyosins from different sources has been assessed in a few studies.²⁵⁻³⁰ The aim of the present study was to investigate whether measurement of IgE levels to shrimp tropomyosin would provide added diagnostic value to currently available methods (ie, SPTs and measurement of shrimp-specific IgE levels by means of fluoroenzyme immunosorbent assay) for identification of shrimp sensitization and prediction of clinically relevant reactions on shrimp ingestion among shrimp-sensitized patients.

METHODS

Patients

We have studied 35 patients with asthma, rhinitis, or both sensitized to *Dermatophagoides pteronyssinus*. Subjects were recruited from the Allergy Clinic of the Department of Clinical Immunology and Allergy of the Hospital das Clínicas, University of São Paulo Medical School, between April 2007 and January 2008. The study was approved by the local research ethics committee.

Subjects responded to a questionnaire that included inquiries regarding their history of reaction to shrimp, symptoms at presentation, and dates of the last reaction, last ingestion of shrimp, or both. For those without a history of food allergy, the current frequency of shrimp consumption was queried.

SPTs

SPTs were performed in duplicate on both arms by a single experienced investigator (A.C.Y.) using extracts of inhalant allergens (IPI-ASAC, Alicante, Spain) and shrimp (1:10 wt/vol; Hollister-Stier Laboratories, Spokane, Wash). The Hollister-Stier 1:10 wt/vol shrimp extract is prepared from boiled *Penaeus setiferus* (large "prawn" from Gulf of Mexico) with a protein content of 54,000 to 286,000 PNU/mL. A positive SPT response was defined as the presence of a wheal with a mean diameter of at least 3 mm greater than that elicited by the negative control accompanied by erythema 15 minutes after application of the extract.

Measurement of IgE antibodies to shrimp and shrimp tropomyosin

Serum IgE antibodies to shrimp were measured by using the ImmunoCAP 100 system (Phadia, São Paulo, Brazil). The ImmunoCAP f24 shrimp solid phase is prepared by using 4 different shrimp species: *Pandalus borealis* (boiled, frozen); *Penaeus monodon*, *Metapenaeopsis barbata*, and *Metapenaeus joyneri* (raw, frozen). Levels of greater than 0.7 kU_A/L were considered positive. Quantitation of IgE to shrimp tropomyosin was performed by using a chimeric ELISA (Indoor Biotechnologies, Inc, Charlottesville, VA), as

previously described.³¹ Briefly, microtiter plates were coated with mAb 1A6 (anti-tropomyosin) at a concentration of 1 µg per well in carbonate-bicarbonate buffer, pH 9.6. After washing with PBS-Tween, shrimp extract (1:20 wt/vol; Greer Laboratories, Lenoir, NC) diluted 1:13,000 was added. The Greer 1:20 wt/vol glycerinated shrimp extract is produced from raw (uncooked) *Penaeus aztecus* and *Penaeus setiferus*, with a Bradford protein content of 2 to 2.5 mg/mL. Subsequently, subjects' sera were added at 1:10 dilution. Detection of IgE to shrimp tropomyosin was carried out with biotinylated goat anti-human IgE (Kirkegaard & Perry Laboratories, Gaithersburg, Md), followed by addition of streptavidin-peroxidase at 1:1,000 dilution and measurement of optical density at 405 nm. For all steps, 1% BSA-PBS-Tween was used as diluent. The assay was quantitated in kilounits per liter by using a control curve constructed with the chimeric antibody 2B12, as previously described.³¹

Oral food challenges

DBPCFCs were performed in all subjects with a positive history of reactions to shrimp, and responses were considered positive if objective signs of allergy were observed. If the double-blind challenge result was negative or inconclusive, the patient was given an open oral challenge. Vital signs, including pulse, blood pressure, and peak flow rate, were monitored, and the patient was observed for evidence of signs and symptoms of skin reactions (eg, pruritus, rash, urticaria, and angioedema), gastrointestinal symptoms (eg, nausea, vomiting, and abdominal pain), respiratory symptoms (eg, wheezing, shortness of breath, and stridor), or symptoms that suggested cardiovascular difficulties (eg, dizziness, weakness, and a sense of doom/fear). None of the subjects had clinical symptoms of atopic disease at the time of the oral challenge. For DBPCFCs, capsules were prepared containing 500 mg of lyophilized *Litopenaeus vannamei* shrimp or an equivalent dose of lactose (placebo). *L. vannamei* is among the 3 most consumed shrimp species in Brazil, which also include *Penaeus brasiliensis* and *Xiphopenaeus kroyeri*. Capsules were administered every 20 minutes, with gradual increases in doses (first dose, 500 mg; second dose, 500 mg; third dose, 1 g; fourth through sixth doses, 2 g; maximum dose, 8 g). Subjects who did not present a positive response (ie, reproduction of objective symptoms) were administered an open challenge of 12 whole cooked shrimp (equivalent to 30 g) after an interval of at least 48 hours. The challenge procedure was halted if a definitive reaction was provoked. To expedite the study, subjects who had no previous history of reactions to shrimp underwent the open challenge first. In the case of a positive or inconclusive outcome, diagnostic confirmation of shrimp allergy was carried out by means of DBPCFC. Equipment and medications for emergency attendance, such as adrenalin, corticosteroids, antihistamines, and β_2 -agonists, were always available. The procedure was performed in a hospital environment, and patients remained under observation for 2 hours after the last dose. For the purpose of the present study, subjects with a positive SPT response, serum IgE antibodies, or both to shrimp were defined as sensitized to shrimp.

Statistical analysis

Statistical analysis was performed with SPSS for Windows version 13.0 (SPSS, Inc, Chicago, Ill). Two-by-two tables were used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and efficiency. Sensitivity was defined as the proportion of true-positive results detected among allergic subjects, and specificity was defined as the proportion of true-negative results detected among nonallergic subjects. The PPV describes the proportion of allergic subjects among those having positive test results, and the NPV describes the proportion of nonallergic subjects among those having negative test results. Efficiency was defined as the proportion of true-positive and true-negative results detected among the total number of tests.

RESULTS

Ages ranged from 16 to 50 years (median, 30 ± 11.5 years), with 10 male and 25 female subjects. Twenty subjects presented with asthma and allergic rhinitis, 14 had rhinitis only, and 1 had

TABLE I. Demographic data, clinical history, symptoms on challenge, skin tests to shrimp, and IgE measurements in patients with positive DBPCFC results to shrimp (shrimp allergy)

No.	Sex	Age (y)	Clinical presentation	History of reactions to shrimp	Symptoms on challenge	SPT to shrimp	IgE to shrimp by CAP (kU _A /L)	IgE to shrimp tropomyosin by ELISA (kU/L)
1	F	18	Asthma, AR	O, Sw, T, U, A, W	O, Sw, T, U, A	Pos	41.8	291.7
2	F	50	Asthma, AR	O, Sw, P, A, NC, R	O, Sw, P, A, T	Pos	15.1	52.1
3	F	22	Asthma, AR	O, Sw, P, S, AP	O, A	Pos	4.64	10
4	M	18	Asthma, AR	O, Sw, U, A, W	O, Sw, U, T, P	Pos	5.53	15.4
5	F	29	Asthma, AR	Neg	O, Sw, T, A	Pos	0.63	4.2
6	F	39	Asthma, AR	O, A, NC, R, S, P, T	O, A, NC, R, S, U	Neg	0.74	<0.4
7	F	28	Asthma, AR	A, N, O, Sw	A, N	Neg	<0.35	<0.4

A, Angioedema; AP, abdominal cramp or pain; AR, allergic rhinitis; NC, nasal congestion; N, nausea; Neg, negative; O, oral pruritus; P, pruritus; Pos, positive; R, rhinorrhea; S, sneezing; Sw, difficulty swallowing; T, throat tightness; U, urticaria; W, wheezing.

asthma only. Eight subjects reported a prior history of immediate clinical manifestations after shrimp ingestion, and 3 of the 8 also reported itching on the hands when they touched raw shrimp. Twenty-seven subjects had never knowingly had a reaction to shrimp.

Sensitization to shrimp and shrimp tropomyosin

SPT responses to shrimp were positive in 15 subjects; 12 subjects had levels of IgE to shrimp of greater than 0.70 kU_A/L. Seven subjects had positive responses to both tests. A total of 20 subjects were therefore considered sensitized to shrimp. Of those, 7 (35%) had detectable IgE to shrimp tropomyosin. No IgE to shrimp tropomyosin was detected among patients without shrimp sensitization.

Clinical outcome of oral challenges

Oral challenges confirmed the diagnosis of shrimp allergy in 6 of the 8 subjects with positive histories: 2 during the DBPCFC and 4 during the open challenge. In the 2 subjects who reported previous reactions to shrimp and had negative DBPCFC and open challenge results, reactions had occurred more than 5 years ago, and reactivity on SPTs and measurement of specific IgE to shrimp or shrimp tropomyosin were negative. Oral open challenge results with shrimp were negative in 26 of the 27 subjects who did not have a previous history of shrimp reaction. One subject with a negative history showed symptoms during the open challenge, with confirmation obtained by means of DBPCFC. Overall, 7 subjects were given diagnoses of shrimp allergy: 6 subjects with a positive history and 1 shrimp-sensitized subject with a negative history. Symptoms reported by history were compared with those elicited by means of oral challenge (Tables I and II). Only 1 subject described a late-phase reaction, with recurrence of symptoms few hours after remission.

Comparison of IgE responses to shrimp with outcomes of oral food challenges

Of the 15 positive SPT responses to shrimp, only 5 were confirmed by means of oral challenge, comprising 10 positive SPT responses without clinical correlation. In contrast, 2 of the negative SPT responses resulted in a positive outcome in the oral challenge with shrimp. Five of the 12 subjects who had positive results for IgE to shrimp had a positive outcome in the oral

challenge, comprising 7 positive shrimp-specific IgE test results without clinical correlation (Table III). On the other hand, 2 of the subjects with negative results for shrimp-specific IgE had positive outcomes in the oral challenge. Five of the 7 subjects with positive IgE to shrimp tropomyosin had positive shrimp challenge results, whereas only 2 were tolerant (Table III).

The results for sensitivity, specificity, PPV, NPV, and efficiency of SPTs, measurements of IgE to shrimp, and measurement of IgE to shrimp tropomyosin are shown on Table IV. Specificity of measurement of IgE to shrimp tropomyosin was greater than that of measurement of IgE to shrimp and the SPT (92.8% vs 75% vs 64.2%, respectively). PPVs were low for SPTs (33.3%) and measurement of IgE to shrimp (41.6%) compared with PPVs for IgE to shrimp tropomyosin (71.4%). The NPVs were similar in all 3 tests, ranging from 90% to 92.8%. Regarding diagnostic efficiency, measurement of IgE to shrimp tropomyosin was also found to be superior (88.5%) to measurement of IgE to shrimp (74.2%) and SPTs (65.7%). Moreover, the likelihood ratio for a positive oral challenge was higher for measurement of IgE to shrimp tropomyosin compared with that of shrimp skin testing or measurement of IgE to shrimp (10, 2, and 2.85, respectively; Table IV).

Clinical outcomes of oral food challenges in relation to a clinical history of reactions to shrimp ingestion and to detection of IgE to shrimp tropomyosin are shown in Fig 1.

DISCUSSION

In the present study we demonstrated that measurements of IgE levels to shrimp tropomyosin provided added diagnostic value to the current available methods for confirming shrimp allergy in patients with suspected reactions on ingestion of shrimp. In particular, using measurement of IgE to shrimp tropomyosin improved diagnostic specificity, PPV, and efficiency for prediction of a clinically relevant reaction to shrimp. Interpretation of our results should be done in light of clinical history. We found that a positive history coupled with a positive test result for IgE to shrimp tropomyosin resulted in positive oral challenge results 100% of the time. Likewise, a negative history coupled with a negative test result for IgE to shrimp tropomyosin was always associated with a negative challenge result. Twenty-seven patients provided no history of reactions to shrimp ingestion, and of those, 26 had negative oral challenge results to shrimp; only 2 had detectable IgE to shrimp tropomyosin compared with 7 who had IgE to shrimp, 10 who had positive skin test responses to shrimp, and 14 who had IgE to shrimp, positive skin test responses, or both.

TABLE II. Demographic data, clinical history, symptoms on challenge, skin tests to shrimp, and IgE measurements in patients with negative challenge results to shrimp (shrimp tolerant)

No.	Sex	Age (y)	Clinical presentation	History of reactions to shrimp	Symptoms on challenge	SPT to shrimp	IgE to shrimp by CAP (kU _A /L)	IgE to shrimp tropomyosin by ELISA (kU/L)
1	F	21	Asthma, AR	Neg	No	Pos	1.54	4.6
2	M	13	AR	Neg	No	Pos	1.19	4.58
3	F	19	AR	Neg	No	Pos	1.33	<0.4
4	F	31	Asthma, AR	Neg	No	Pos	1.09	<0.4
5	F	24	AR	Neg	No	Pos	0.46	<0.4
6	F	57	Asthma, AR	Neg	No	Pos	0.37	<0.4
7	M	40	Asthma	Neg	No	Pos	<0.35	<0.4
8	F	47	Asthma, AR	Neg	No	Pos	<0.35	<0.4
9	F	32	Asthma, AR	Neg	No	Pos	<0.35	<0.4
10	F	17	AR	Neg	No	Pos	<0.35	<0.4
11	M	50	AR	Neg	No	Pos	<0.35	<0.4
12	F	47	A	Neg	No	Neg	2.45	<0.4
13	M	25	Asthma, AR	Neg	No	Neg	1.98	<0.4
14	M	19	AR	Neg	No	Neg	1.53	<0.4
15	M	35	AR	U, A	No	Neg	<0.35	<0.4
16	F	33	Asthma, AR	N, AP, T	No	Neg	<0.35	<0.4
17	M	42	AR	Neg	No	Neg	<0.35	<0.4
18	F	17	AR	Neg	No	Neg	<0.35	<0.4
19	F	25	Asthma, AR	Neg	No	Neg	<0.35	<0.4
20	F	17	AR	Neg	No	Neg	<0.35	<0.4
21	F	16	AR	Neg	No	Neg	<0.35	<0.4
22	F	39	Asthma, AR	Neg	No	Neg	<0.35	<0.4
23	M	37	Asthma, AR	Neg	No	Neg	<0.35	<0.4
24	F	26	AR	Neg	No	Neg	<0.35	<0.4
25	M	35	AR	Neg	No	Neg	<0.35	<0.4
26	F	37	AR	Neg	No	Neg	<0.35	<0.4
27	F	20	Asthma, AR	Neg	No	Neg	<0.35	<0.4
28	F	25	Asthma, AR	Neg	No	Neg	<0.35	<0.4

A, Angioedema; AP, abdominal cramp or pain; AR, allergic rhinitis; F, female; M, male; N, nausea; Neg, negative; Pos, positive; T, throat tightness; U, urticaria.

TABLE III. IgE reactivity to shrimp allergens in patients with shrimp allergy and shrimp-tolerant patients

Parameter	Shrimp allergy* (n = 7)	Shrimp tolerance† (n = 28)
Positive IgE to shrimp, no. (%)‡	5 (71.4)	7 (25)
Range (kU _A /L)	0.74–41.8	1.09–2.45
Positive SPT response to shrimp extract, no. (%)	5 (71.4)	10 (35.7)
Positive IgE result to shrimp and/or positive SPT response to shrimp extract, no. (%)	6 (85.7)	14 (50)
Positive IgE result to shrimp tropomyosin, no. (%)§	5 (71.4)	2 (7.1)
Range (kU/L)	4.2–291.7	4.58–4.6

*Positive DBPCFC result.

†Negative open challenge result (26 patients) or negative open challenge and DBPCFC results (n = 2).

‡CAP >0.70 kU_A/L.

§ELISA >0.4 kU/L.

IgE reactivity to foods on skin testing or CAP without clinical correlation has been attributed to sensitization to cross-reacting allergens in other foods or as a result of primary exposure to allergens on sites different from the gastrointestinal tract.¹² In the case of shrimp, it could be hypothesized that cross-reactive responses to tropomyosin, an invertebrate panallergen, could be acquired through exposure to tropomyosins present in other

foods, parasites, or mites and cockroach through the inhalant route.²³ However, our results do not support this hypothesis. Among the group of patients with mite allergy with asthma, rhinitis, or both evaluated in the present study with a high frequency of reactions to shrimp on skin testing and CAP without clinical correlation, IgE to shrimp tropomyosin was found in a much smaller proportion of patients.

Interestingly, Soeria-Atmadja et al³² have performed a multivariate data analysis of IgE antibody serum concentrations of 1,011 subjects to 89 allergen extracts and identified 12 stable clusters. Reactivity to shrimp and mussels was clustered with cockroach but unexpectedly not with house dust mites, supporting the view that mite tropomyosins (Der p 10 and Der f 10) are less important components in mite sensitization. One possibility is the presence of IgE reactivity to additional cross-reactive allergens in subjects who are cosensitized to dust mites and shrimp. Alternatively, certain structures, including cross-reacting carbohydrate determinants, which are identified primarily in pollens and insect venoms, are known to give rise to specific IgE antibodies without consistent relation to clinical symptoms; however, the presence of cross-reacting carbohydrate determinants in shrimp extracts has not been reported.³³ Taken together, these observations suggest that IgE to shrimp tropomyosin is mainly linked to shrimp allergy and not to clinically irrelevant IgE cross-reactivity.

The use of additional methods, including intradermal tests, the prick-to-prick method, skin tests with fresh material, and titration skin tests, could have improved the sensitivity or specificity of

TABLE IV. Predictive capacity of SPTs, IgE to shrimp, and IgE to shrimp tropomyosin for positive oral food challenge results

	SPT	IgE to shrimp	IgE to shrimp tropomyosin
Sensitivity (95% CI)	71.4% (30.2% to 94.8%)	71.4% (30.2% to 94.8%)	71.4% (30.2% to 94.8%)
Specificity (95% CI)	64.2% (44.1% to 80.6%)	75% (54.7% to 88.5%)	92.8% (75% to 98.7%)
PPV (95% CI)	33.3% (12.9% to 61.3%)	41.6% (16.4% to 71%)	71.4% (30.2% to 94.8%)
NPV (95% CI)	90% (66.8% to 98.2%)	91.3% (70.4% to 98.4%)	92.8% (75% to 98.7%)
Efficiency	65.7%	74.2%	88.5%
Positive likelihood ratio* (95% CI)	2 (1.01 to 3.95)	2.85 (1.29 to 6.32)	10 (2.42 to 41.17)

*Positive likelihood ratios: greater than 10, large and often conclusive increase in the likelihood of disease; 5 to 10, moderate increase in likelihood of disease; 2 to 5, small increase in likelihood of disease; 1 to 2, minimal increase in likelihood of disease; 1, no change in likelihood of disease.

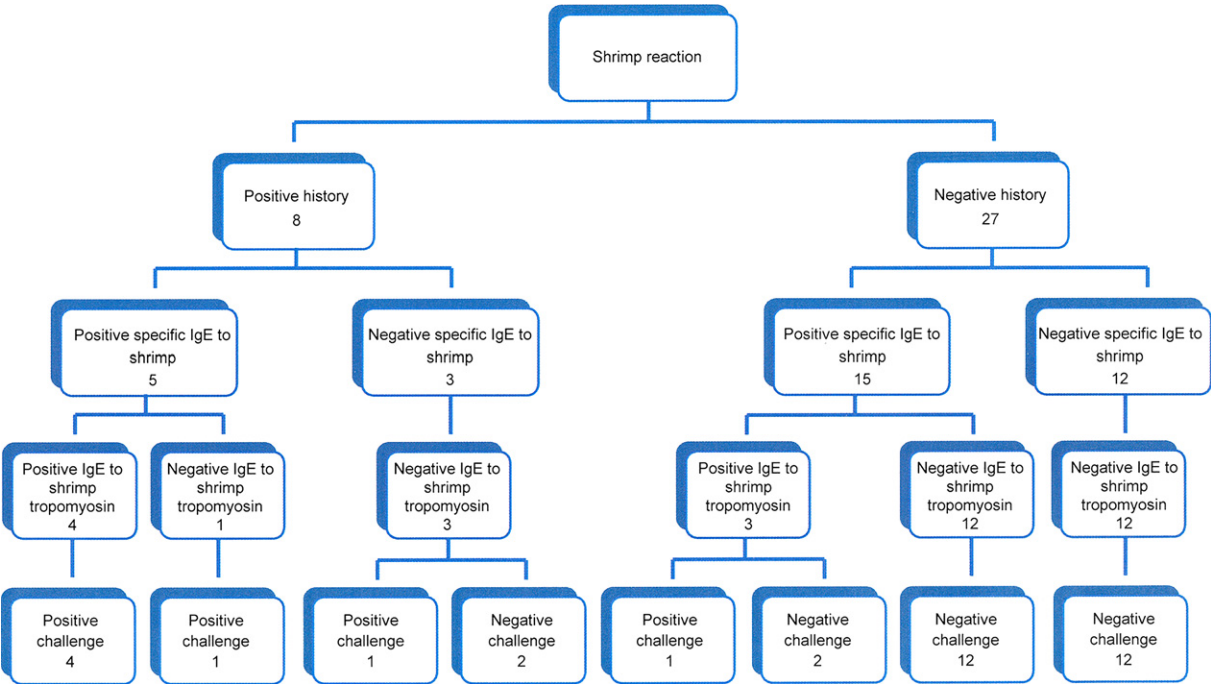


FIG 1. Outcomes of oral food challenges according to history of reactions to shrimp and the presence of IgE to shrimp (determined by means of SPTs, shrimp ImmunoCAP, or both) and shrimp tropomyosin.

skin testing in the present study. However, we aimed to have our results applied as close as possible to the diagnostic routine of the practicing allergist. Intradermal skin tests with foods have not been recommended by a joint practice parameter on allergy diagnostic testing by the American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma, and Immunology because of an unacceptably high false-positive rate and the potential risk of systemic reactions, which could be avoided in the majority of cases by prescreening with prick test.³⁴ Likewise, the safety and predictive values of prick-to-prick testing with shrimp have not been determined. Preparation of fresh shrimp extracts often requires blending, freezing at -70°C , lyophilization, high-speed centrifugation, and dialysis, which would not be available in most allergist's offices. Titration of skin test reactivity with different concentrations of shrimp extract could discriminate clinical reactors from the nonreactors. However, a major issue in establishing cut-off levels would be the heterogeneity of extracts. The present study highlighted differences in shrimp extracts for skin testing and IgE measurements, including selection of a variety of shrimp species and use of boiled, uncooked raw material, or both, which could account for discrepancies in *in vivo* and *in vitro* test results.

It is possible that patients who were sensitized to shrimp and did not react to tropomyosin might have IgE reactivity to other allergens, including the myosin light chain shrimp allergen Lit v 3 (homologous to cockroach Bla g 8),³⁵ the arginine kinase shrimp allergens Pen m 2 and Lit v 2 (a possible new class of invertebrate panallergens),^{36,37} and a sarcoplasmic calcium-binding protein shrimp allergen.³⁸ Although measurement of IgE to shrimp tropomyosin seems to be more effective than other current diagnostic methods, it cannot be the only diagnostic assay to be used because of the possible reactivity to shrimp allergens other than tropomyosin. In this regard the performance of a panel of purified natural, recombinant, or both shrimp allergens on component-resolved diagnosis of shrimp allergy could be investigated. Previous studies using this approach for diagnosis of allergy to peanut, cherry, apple, and peach have shown increased sensitivity over routine methods, as well as the possibility to study differences in sensitization profiles of patients living in different locations and to predict more severe reactions.^{15,19}

In the present study a positive history was not confirmed by oral challenges in 2 patients; both of them had negative skin test results and nondetectable IgE antibodies to shrimp or shrimp tropomyosin. These data strengthen the necessity of caution when

judging personal reports. Such discordant results might occur as a result of false etiologic associations, mainly when symptoms appear after ingestion of an assortment of foods. Adverse reactions by non-immune-mediated mechanisms (eg, toxins or additives in crustaceans) or by IgE-mediated reactivity to the parasite *Anisakis* species must also be considered.³⁹⁻⁴¹ Furthermore, the development of tolerance can occur in subjects who have seafood allergy.^{42,43}

One important issue is whether the presence of an IgE response to shrimp or shrimp tropomyosin would be associated with an increased risk to subsequently have shrimp allergy, particularly with severe reactions. One subject in our study with no prior history of reactions to shrimp was given a diagnosis of shrimp allergy after the oral challenge. It is known that allergy to shellfish is more common in adults,^{1,43} and a history of previous consumption without triggering symptoms is not unusual. This patient was sensitized to shrimp and presents IgE to shrimp and shrimp tropomyosin. The answer to whether IgE to shrimp tropomyosin could predict subsequent reactions on shrimp ingestion could only be obtained by performing a prospective study.

In conclusion, diagnosis of shrimp allergy is often a challenge for clinicians, particularly when facing patients with inconsistent reactions, mild subjective symptoms, or no prior history of reaction on shrimp ingestion but with a positive skin test or CAP result to shrimp obtained during routine workup for allergies. Our results show evidence that testing for IgE to shrimp tropomyosin is a new option for the diagnostic approach to seafood allergy, which could help clinicians in terms of issuing recommendations for shrimp avoidance and providing advice on risks. Although no method was reliable enough to eliminate the oral challenge, evaluation of clinical history coupled with specific IgE levels was very effective in predicting the outcome of oral challenges. In particular, IgE to shrimp tropomyosin showed value in revealing clinical response to shrimp unexpectedly in patients who were perceived to be primarily mite allergic. We believe that because of the greater specificity and PPV demonstrated in this study, we will be able to optimize the diagnosis of allergy to shrimp.

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Clinical implications: Our results suggested that detection of IgE to shrimp tropomyosin in subjects allergic to dust mites is directly linked to the presence of shrimp allergy and could improve the diagnosis of shrimp allergy.

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